

claims 2 and 4-6, which were allowed over one year ago. (see Paper No.: 28 at 9 and Office Action Summary), without providing any acknowledgement of the PTO's previous determination let alone an explanation of why the PTO determination was clear error as required by the PTO's own rules (see MPEP §706.04).

As the Examiner can appreciate, it is wasteful of PTO and applicant time and resources to revisit settled issues in the absence of a finding of clear error in previous PTO determinations. Accordingly, it is respectfully requested that the Examiner reconsider and withdraw those rejections that revive settled issues, namely the allowance of claims 2 and 4-6 as well as certain §112 rejections identified further below.

The current drawings have been replaced with the Substitute Drawings (sheets 1-4) attached hereto as Exhibit 1. Figures 1-4 have been reformatted to correct the sizes of the margins in order to conform to the requirements of 37 C.F.R. §1.84(g), and all of the numbers and reference characters in figures 3 and 4 have been reproduced in a plain and legible manner in order to conform to the requirements of 37 C.F.R. §1.84(p).

In accordance with 37 C.F.R. §121(d) and MPEP §608.02(p), a TRANSMITTAL OF PROPOSED DRAWING CORRECTIONS with attached corrected drawings is being submitted concurrently herewith.

The specification has been amended to insert a paragraph reciting the full address of the American Type Culture Collection (ATCC) as requested by the Examiner. (See Paper No. 33 at 2).

Claim 1 has been amended to recite the entire phrase for which the abbreviation "DAPA" is used, --diaminopelargonic acid--. Claim 13 also has been amended to recite the entire phrase for which the abbreviation "SAM" is used, --S-adenosylmethionine--. These clarifying amendments are formal in nature and do not narrow the scope of the claims in any manner.

Claims 1 and 6 have been amended to recite that lysine, a lysine analog, or a lysine precursor is added --to provide a concentration of at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step--. Claims 17 and 18 have been amended to recite that the exogenously added lysine or lysine analog --provides a concentration of at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step--. Support for these amendments is found in original claims 1 and 6, and in the specification at, for example, page 19, lines 4-7, page 21, lines 5-8, page 22, lines 4-7, and Tables 3, 5A, 5B, 6 and 8. See *In re Gardner*, 177 USPQ 396, 397 (CCPA 1973) and MPEP §§608.01 (I) and (o). These clarifying amendments are formal in nature and do not narrow the scope of the claims in any manner.

Claim 2 has been amended to recite --wherein the lysine biosynthetic pathway is deregulated in-- said bacterium. Claims 11 and 21 have been amended to recite --of the *bioA* gene is deregulated in said bacterium--. These clarifying amendments are formal in nature and do not narrow the scope of the claims in any manner.

It is submitted that no new matter has been introduced by the foregoing amendments. Approval and entry of the amendments is respectfully solicited.

Objections

The specification was objected to as not being in compliance with 37 C.F.R. §1.809(d). (Paper No. 33 at 2). In an effort to further prosecution, the specification has been amended to include the name and address of the depository as suggested by the Examiner. Accordingly, the objection is rendered moot and should be withdrawn.

The drawings were objected to by the draftsman as containing certain informalities. (*Id.* at 3). In an effort to further prosecution, drawing corrections are enclosed herewith as Exhibit 1. In view of the foregoing, withdrawal of the drawing objections respectfully is requested.

Claims 1-4, 11, 13 and 21 were objected to for reciting "DAPA", "SAM", or "bioA". (Paper No. 33 at 3) In making the objection, the Examiner asserted that "[a]bbreviations unless otherwise obvious and/or commonly used in the art, should not be recited in the claims without at least once reciting the entire phrase for which the abbreviation is used." (*Id.*).

Initially, we note that the term "*bioA*" refers to the *bioA* gene and that this is **not an abbreviation** and represents standard terminology for the recitation of a gene. Further, the Examiner seems to have correctly ascertained that the term "*bioA*" refers to "the gene encoding DAPA aminotransferases." (Paper No. 33 at 5).

With a view towards furthering prosecution, however, claims 11 and 21 have been amended to recite --expression of the *bioA* gene--. With respect to the "DAPA" and "SAM" abbreviations, claim 1 has been amended to recite the entire phrase for which the abbreviation "DAPA" is used and claim 13 has been amended to

recite the entire phrase for which the abbreviation "SAM" is used. In view of the foregoing, it is respectfully submitted that the objections have been rendered moot and should be withdrawn.

§112, Second Paragraph Rejection

Claims 1-22 were rejected under 35 U.S.C. §112, second paragraph. (Paper No. 33 at 3). In making the rejection, the Examiner asserted that "[c]laims 1, 6, 17 and 18... are indefinite in the recitation of 'precursor is exogenously added to the culture and totals at least 10 mmoles per liter' as it is unclear what totals 10 mmoles per liter.'" (*Id.* at 4).

With a view towards furthering prosecution, claims 1, 6, 17 and 18 have been amended as set forth above to recite that it is the concentration of lysine, lysine analog or lysine precursor in the culture that totals at least 10 mmoles per liter. Accordingly, it is respectfully submitted that the rejection is rendered moot and should be withdrawn.

In making the rejection, the Examiner further asserted that "[c]laims 2, 11, 21... are indefinite in the recitation of 'bacterium is deregulated with respect to lysine production' or 'bacterium is deregulated with respect to at least one biotin synthetic pathway' as it is unclear what the meaning of the term 'deregulated' is as it relates to a bacterium." (*Id.*).

With a view towards furthering prosecution, and in accordance with the Examiner's recommendation, claim 2 has been amended to recite --the lysine biosynthetic pathway is deregulated in-- said bacterium and claims 11 and 21 have

been amended to recite --wherein at least one biotin synthetic pathway step in addition to expression of the *bioA* gene is deregulated in said bacterium--. Accordingly, it is respectfully submitted that the rejection is rendered moot and should be withdrawn.

§112, First Paragraph Rejections

1. Written Description

Claims 1-22 were rejected under 35 U.S.C. §112, first paragraph. (Paper No. 33 at 5). In making the rejection, the Examiner asserted that "[t]he specification discloses only a few species of the genera of microorganisms, lysine and SAM-utilizing DAPA aminotransferases, enzymes involved in the regulation of lysine and biotin synthesis and mutations to said enzymes, to practice a method for producing biotin vitamers which is insufficient to put one of ordinary skill in the art in possession of all attributes and features of the claimed method." (*Id.* at 6).

As is well accepted, there is a **strong presumption** that an adequate written description of the claimed invention is present in an application as filed. See *In re Werthheim*, 191 USPQ 90, 97 (CCPA 1976); and MPEP §2163(II)(A). Further, an applicant may show possession of the claimed invention by describing it using descriptive means such as, for example, words, structures, figures, diagrams and formulas. See MPEP §2163(I).

The present specification provides **specific definitions** for the terms "lysine-utilizing DAPA aminotransferase" (page 6, lines 1-6) and "SAM-utilizing DAPA aminotransferase" (page 6, lines 3-8). Accordingly, it is respectfully submitted that in view of these definitions alone, one of skill in the art at the time the application was filed

would readily understand that the inventors were in possession of these aspects of the claimed invention.

Furthermore, the written description requirement for a claimed genus may be satisfied by sufficient description of a **representative number of species**. See *Regents of University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997); and MPEP § 2163 (II)(A)(3)(a)(ii). In fact, there are situations where even one species can adequately support a genus. See *Rasmussen*, 211 USPQ 323, 326-27 (CCPA 1981).

As the Examiner acknowledges, "the specification discloses *B. subtilis* or *E. coli* wherein the gene encoding DAPA amino transferases (*bioA*) from *B. subtilis*, *E. coli* or *S. marcescens* is expressed." (Paper No.33 at 5). The specification also discloses how to identify a lysine-utilizing DAPA aminotransferase. (See page 5, lines 21-27). *Tested*

Further, the specification discloses the use of strains **engineered** to overexpress *bioA* off of genetic constructs (see page 11, line 31 to page 12, line 8 and page 18 line 26 to page 19 line 4), as well as strains **engineered** to express multiple DAPA-amino transferases, including SAM- and lysine-utilizing DAPA-amino transferases (see page 6, line 27 to page 7, line 2 and page 19, line 29 to page 20, line 31).

The specification also discloses a general enzyme assay for identifying enzymes that are DAPA aminotransferases (see page 13, line 5 to page 15, line 29), as well as assays for identifying enzymes that are lysine-utilizing (see page 14, line 28 to

page 15, line 29) and SAM-utilizing (page 13, line 26 to page 14, line 27) DAPA aminotransferases.

The specification also discloses how to deregulate lysine production (see page 7, lines 17-30), identifies a comprehensive textbook regarding the biosynthesis of lysine and its regulation (see citation to Hermann & Somerville, Amino Acids: Biosynthesis and Genetic Regulation, on page 7, lines 25-30) and provides a diagram of the pathway involved in lysine production (see Figure 4).

Further, the specification discloses mutations that deregulate lysine production (see page 12 line 29 to page 13, line 4) as well as bacteria having deregulated lysine production (see page 22, lines 14-26). Also, known strains of *Brevibacter* and *Corynebacter* that have deregulated lysine production are disclosed (see page 22, lines 17-19) and known mutants of *B. subtilis* that have deregulated lysine production (see page 22, line 27 to page 23, line 14 and Table 7) are described. Further, the isolation of a mutant bacteria deregulated for lysine production (see page 23, line 15 to page 24, line 15) is disclosed.

The deregulation of a biotin synthetic step is also disclosed in the specification (see page 7, line 31 to page 8, line 5), as are strains engineered to be deregulated for a biotin synthetic step (see page 3, line 30 to page 4, line 6; page 18, line 32 to page 19, line 4; and page 19, line 29 to page 20, line 31) and methods for deregulating biotin biosynthesis (see page 3, line 30 to page 4, line 6).

Thus, based on:

- (1) the specific definitions for the terms "lysine-utilizing DAPA aminotransferase" and "SAM-utilizing DAPA aminotransferase" provided in the specification;
- (2) the detailed description of engineered strains that overexpress lysine-utilizing DAPA aminotransferase, alone and in combination with the expression of SAM-utilizing DAPA aminotransferases, wherein the DAPA aminotransferases themselves originate from a variety of strains;
- (3) the disclosure of engineered strains derived from a variety of bacterial backgrounds that are deregulated in the production of lysine;
- (4) the identification of known *B. subtilis* mutants that are deregulated for lysine production;
- (5) the description of how to deregulate lysine production;
- (6) comprehensive background information regarding lysine production, including a diagram of the pathway for lysine production;
- (7) the detailed description of the isolation of a mutant that is deregulated for lysine production;
- (8) the description of strains deregulated for a biotin synthetic step in addition to *bioA*;
- (9) the description of the deregulation of a biotin synthetic step;

(10) the disclosed assays for identifying a lysine-utilizing DAPA aminotransferase; and

(11) the disclosed assays for measuring the enzymatic activity of lysine-utilizing and SAM-utilizing DAPA aminotransferases,

it is respectfully submitted that the Applicants were in possession of the full scope of the claimed invention at the time the application was filed.

Moreover, a proper written description analysis requires an analysis of the understanding of an ordinarily skilled artisan at the time of the invention. See MPEP § 2163(II)(A)(2); see also *Wang Labs. v. Toshiba Corp.*, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993). As noted above, the specification provides ample background information detailing the knowledge in the art regarding the biosynthesis of biotin vitamers in numerous bacteria, such as, for example, *E. coli*, *Salmonella Typhimurium*, *B. subtilis*, and *B. sphaericus*. (See page 1, line 8 to page 4, line 6). In addition, the specification describes genetic aspects of the *bio* operon in *E. coli*, *B. subtilis*, and *B. sphaericus*. (See page 2, line 19 to page 3, line 30). In view of the foregoing, it is respectfully submitted that the Examiner has not properly taken into account this background information in determining whether the inventors were in possession of the claimed invention at the time the application was filed. Accordingly, for this reason also the rejection should be withdrawn.

We further note that possession may be shown by description of an actual reduction to practice of the claimed invention and that an actual reduction to practice may be shown by describing the deposit of biological materials in accordance with 37 C.F.R. 1.801 *et seq.* See MPEP § 2163(I). Further, the Federal Circuit Court recently

confirmed that the deposit of biological materials is sufficient to comply with the written description requirements. See *Enzo Biochem, Inc. v. Gen-probe, Inc.*, 63 USPQ2d 1609, 1614 (Fed. Cir. 2002) (“[w]hile deposit in a public depository most often has pertained to satisfaction of the enablement requirement, we have concluded that **reference in the specification to a deposit may also satisfy the written description requirement....**” (emphasis added)).

Here, strain **BI282** is a **deposited strain** that is described in the specification as genetically engineered to be deregulated in one biotin synthetic pathway in addition to *bioA* expression and to overexpress *bioA*. (See page 13, line 26 to page 14, line 1 and page 3, line 30 to page 4, line 6). We respectfully refer the Examiner to pages 3-4 of the June 1, 1999 RESPONSE TO OFFICE ACTION INCLUDING AMENDMENT, which notes that strain BI282 was deposited with the American Type Tissue Culture Collection (“ATCC”) under the terms of the Budapest Treaty, and to page 25 of the same paper wherein the specification was amended to reflect the deposit.

Strain **BI603** is another **deposited strain** (see page 24, line 16 to page 25, line 25) that is described in the specification as genetically engineered to be deregulated in one biotin synthetic pathway in addition to *bioA* expression and to overexpress *bioA* (see page 18, line 32 to page 19, line 4).

Also, strains **BI90** and **BI96** are **deposited strains** (see page 24, line 16 to page 25, line 25) that are described in the specification as genetically engineered to carry both SAM-utilizing and lysine-utilizing DAPA aminotransferases and to be

deregulated in one biotin synthetic pathway in addition to *bioA* expression (see page 19, line 29 to page 20, line 31).

Further, strains **BI641** and **BI642** are **deposited strains** (see page 24, line 16 to page 25, line 25) that are described in the specification as deregulated for lysine production (see page 23, line 15 to page 24, line 15).

In *Enzo*, the deposit of a **single** strain was deemed sufficient for compliance with the written description requirements. Here, **six** different deposited strains conveying possession of all aspects of the claimed invention have been provided. If one deposit was sufficient for the Federal Circuit, surely the deposit of six different microorganisms within the scope of the present claims is sufficient to show possession by the applicants before the PTO. For this reason also, withdrawal of the rejection is respectfully requested.

We further note that the **claims are drawn to a method**, and not to compositions of matter. Accordingly, the written description analysis is focused on the **steps** of the method. Thus, we direct the Examiner's attention to the written description analysis in the Revised Interim Written Description Guidelines Training Materials ("Training Materials") for claims drawn to a composition and those that are drawn to a method (*i.e.*, compare Example 6 drawn to an isolated gene and Example 18 drawn to a method of producing a protein). The method claim analyzed in Example 18 of the Training Materials and the accompanying analysis of the claim for compliance with the written description requirement is reproduced below in its entirety (emphasis added):

Example 18: Process claim where the novelty is in the method steps.

Specification:

The specification teaches a method for producing proteins using mitochondria from the fungus *Neurospora crassa*. In the method, mitochondria are isolated from this fungus and transformed with a mitochondrial expression vector which comprises a nucleic acid encoding a protein of interest. The protein is subsequently expressed, the mitochondria is lysed, and the protein is isolated. The specification exemplifies the expression of A-galactosidase using the claimed method using a cytochrome oxidase promoter.

Claim:

1. A method of producing a protein of interest comprising;
obtaining *Neurospora crassa* mitochondria,
transforming said mitochondria with an expression vector comprising a nucleic acid that encodes said protein of interest,
expressing said protein in said mitochondria, and
recovering said protein of interest.

Analysis:

A review of the specification reveals that *Neurospora crassa* mitochondrial gene expression is essential to the function/operation of the claimed invention. ***A particular nucleic acid is not essential to the claimed invention.***

A ***search of the prior art*** reveals that the claimed method of expression in *Neurospora crassa* is novel and unobvious.

The claim is drawn to a genus, *i.e.*, any of a variety of methods that can be used for expressing protein in the mitochondria.

There is actual reduction to practice of a single embodiment, i.e., the expression of β -galactosidase.

The art indicates that there is no substantial variation within the genus because there are a limited number of ways to practice the ***process steps*** of the claimed invention.

The single embodiment is representative of the genus based on the disclosure of *Neurospora crassa* mitochondria as a gene expression system, considered along with the level of skill and knowledge in the gene expression art. One of skill in the art would recognize that applicant was in possession of all of the various expression methods necessary to practice the claimed invention.

Conclusion:

The claimed invention is adequately described.

(Revised Interim Written Description Guidelines Training Materials, Example 18, pages 65-66).

It is respectfully submitted that based on the representative analysis set forth above for ***method*** claims as compared to the Examiner's analysis that more closely parallels the analysis of composition claims (see e.g., Example 6 of the Training Materials), the present claims are in full compliance with the written description requirement. For this reason as well, the rejection should be withdrawn.

2. Enablement

Claims 1-22 also were rejected under 35 U.S.C. §112, first paragraph. (Paper No. 33 at 7). In making the rejection, the Examiner asserted that the

specification "does not reasonably provide enablement for practicing the claimed method using **any microorganism** capable of expressing any lysine and/or SAM-utilizing aminotransferase wherein lysine or biotin synthesis is deregulated in any way." (*Id.*). The Examiner acknowledged, however, that the specification is "enabling for a method for the production of biotin vitamers using *E. coli* or *Bacillus subtilis* wherein said microorganisms express *E. coli*, *B. subtilis* or *S. marcescens* DAPA aminotransferases and wherein the lysine or biotin synthesis in *E. coli* or *B. subtilis* is deregulated by mutations in the genes encoding aspartokinase, I, II, III or DAP decarboxylase" (*Id.*).

Initially, we note that the MPEP clearly states that "[t]he goal of examination is to **clearly articulate any rejection early in the prosecution process** so that the applicant has the opportunity to provide evidence of patentability and otherwise reply completely at the earliest opportunity. The **examiner then reviews all the evidence, including arguments and evidence responsive to any rejection before issuing the next Office action.**" MPEP §706.

We direct the Examiner's attention to the Office Action mailed on February 1, 1999, wherein the Examiner acknowledged that the specification is "enabling for a method of producing a biotin vitamer by culturing a bacterium comprising a lysine-utilizing DAPA aminotransferase in an environment enriched for lysine or a lysine analog." (Paper No. 12 at 4). This is precisely what is currently claimed. Thus, this exact issue has already been considered by the PTO and **fully addressed in prior prosecution.**

Further, in the Office Action mailed on February 1, 1999 and in the Office Action mailed on March 15, 2000, claims 11 and 21 were rejected for the very same reasons as the Examiner asserts in the current Office Action regarding guidance in obtaining a bacterium deregulated in at least one biotin synthetic step other than *bioA* expression. (See Paper No. 33 at 7-8, Paper No. 19 at 2 and Paper No. 12 at 5-6). These enablement rejections were fully addressed in the RESPONSE filed on August 8, 2000, ("Response") wherein reference is made to an Examiner's Interview between the Examiner, Dr. John Perkins (a co-inventor) and Kevin Hooper of our offices. As is indicated on page 2 of the Response, the Examiner agreed to withdraw the enablement rejections upon identification of the deposited strains BI282 and BI603, both of which carry a lysine-utilizing DAPA aminotransferase and are deregulated in at least one biotin synthetic step other than *bioA* expression, and strains BI90 and BI96, both of which carry a lysine-utilizing DAPA aminotransferase and a SAM-utilizing DAPA aminotransferase and are also deregulated in at least one biotin synthetic step other than *bioA* expression. As indicated on pages 2-3 of the Response, and as set forth in detail in discussing the written description rejections above, the deposit of these strains under the terms of the Budapest Treaty is described in the specification. (See page 24, line 16 to page 25, line 25 and pages 3-4 of the June 1, 1999 RESPONSE TO OFFICE ACTION INCLUDING AMENDMENT). Accordingly, it is respectfully submitted that the current rejection for lack of enablement regarding bacteria that are deregulated in at least one biotin synthetic step other than *bioA* expression has also been **fully addressed and overcome in prior prosecution.**

In addition, in the Office Action mailed on August 18, 1999, the Examiner rejected claims 9, 10 and 13-22 under the enablement provision of 35 U.S.C. §112. (See Paper No. 17 at 3). In order to overcome the rejection, the Examiner required that "bacterium resistant to S-2-aminoethyl-L-cysteine and the bacterium engineered to produce a SAM-utilizing DAPA aminotransferase must be obtainable by a repeatable method set forth in the specification or **otherwise be available to the public.**" (*Id.*). Accordingly, the Examiner requested the deposit of these strains. (*Id.* at 3-4). We note that the bacterium resistant to S-2-aminoethyl-L-cysteine ("AEC") is a reference to mutants described in the specification that are deregulated for lysine production. (See page 22, line 27 to page 23, line 14). As indicated on pages 3-4 of the RESPONSE TO OFFICE ACTION filed on December 17, 1999, and as set forth in detail in discussing the written description rejections above, the specification discloses the deposit of strains BI641 and BI642, which are mutant strains resistant to AEC and which are deregulated for lysine production (see *Id.*), and the deposit of strains BI90 and BI96, which are strains that express a SAM-utilizing DAPA aminotransferase (see page 19, line 29 to page 20, line 31), under the terms of the Budapest Treaty. (See page 24, line 16 to page 25, line 25). It is respectfully submitted that the current rejection for lack of enablement regarding bacteria that are deregulated for lysine production and bacterium that express a SAM-utilizing DAPA aminotransferase have also been **fully addressed and overcome in prior prosecution.**

We note that the Examiner has not indicated why the admissions made by the previous Examiner regarding enablement are no longer applicable or are clearly erroneous and further, the Examiner has not acknowledged the aforementioned

evidence relevant to enablement that was dutifully submitted during this lengthy prosecution process. Considerable effort and expense has already been incurred on the part of applicants addressing the PTO's previous enablement rejections, which the PTO has admitted overcome the rejections. Accordingly, the present Examiner should give the previous determinations by the PTO full faith and credit and not revisit issues that have been fully examined and resolved. See MPEP §706.04 ("Full faith and credit should be given to the search and **action of a previous Examiner** unless there is clear error in the previous action or knowledge of other prior art."). For these reasons alone, the rejection should be reconsidered and withdrawn.

In an effort to further prosecution, however, we will again revisit the enablement issue and again demonstrate that the **claimed** invention is clearly enabled. In this regard, we note that the Examiner identifies the analysis for enablement - the so-called *Wands* factors - taken from a case involving hybridoma technology for producing hepatitis B-surface antigen determinants, wherein the Federal Circuit reversed the Board (and the Examiner) and held that screening large numbers of hybridoma cell lines looking for a "positive" cell line was **not** undue experimentation even though the expected number of "positive" cell lines would be low compared to the high number of hybridoma cells that had to be screened (thousands or more). *In re Wands*, 8 USPQ2d 1400, 1406-07 (Fed. Cir. 1988).

In remaking the enablement rejection, the Examiner asserted that the specification "does not reasonably provide enablement for practicing the claimed method using **any microorganism** capable of expressing any lysine and/or SAM-utilizing aminotransferase...." (Paper No. 33 at 7).

Initially, we note that the Examiner has misconstrued the claims. The claims are **not** directed to "**any microorganism**." Rather, the claims recite a method of producing a biotin vitamer by culturing a bacterium containing certain identified enzymes. (See e.g., claim 1). It appears that the Examiner has ignored the clear language of the claims and given them an interpretation that is inconsistent with the plain language of the claims and the specification. Because the rejection is based on the wrong claim interpretation, the rejection is insufficient as a matter of fact and should be withdrawn for this reason alone.

It is well accepted that even a "considerable amount" of experimentation is permissible as long as it is merely routine or if the specification provides a reasonable amount of guidance. MPEP §2164.06 and *In re Wands*, 8 USPQ at 1404. Further, as the Federal Circuit Court has recently stated, "[t]he enablement requirement is often more indulgent than the written description requirement." *Amgen Inc. v. Hoechst Marion Roussel, Inc.*, 65 USPQ2d 1385, 1399 (Fed. Cir. 2003).

As discussed in detail above in addressing the written description rejection, based on:

- (1) the specific definitions for the terms "lysine-utilizing DAPA aminotransferase" and "SAM-utilizing DAPA aminotransferase;"
- (2) the detailed description of engineered strains that overexpress lysine-utilizing DAPA aminotransferase, alone and in combination with the expression of SAM-utilizing DAPA aminotransferases, wherein the DAPA aminotransferases themselves originate from a variety of strains;

- (3) the disclosure of engineered strains derived from a variety of bacterial backgrounds that are deregulated in the production of lysine;
- (4) the identification of known *B. subtilis* mutants that are deregulated for lysine production;
- (5) the description of how to deregulate lysine production;
- (6) comprehensive background information regarding lysine production, including a diagram of the pathway for lysine production;
- (7) the detailed description of the isolation of a mutant that is deregulated for lysine production;
- (8) the description of strains deregulated for a biotin synthetic step in addition to *bioA*;
- (9) the description of the deregulation of a biotin synthetic step;
- (10) the disclosed assays for identifying a lysine-utilizing DAPA aminotransferase; and
- (11) the disclosed assays for measuring the enzymatic activity of lysine-utilizing and SAM-utilizing DAPA aminotransferases,

it is respectfully submitted that the specification provides ample guidance, numerous working examples, and identifies six deposited microorganisms that would enable one of skill in the art to practice the claimed invention without undue experimentation. Accordingly, withdrawal of the rejection is respectfully requested for these reasons as well.

§102(b) Rejection

Claims 1, 3, 7, 11-19 and 21-22 were rejected under 35 U.S.C. §102(b) as anticipated by Bower *et al.* (EP-0-635-572-A2) ("Bower(EP)"). (Paper No. 33 at 9).

Bower(EP) discloses genes of the biotin biosynthetic operon of *Bacillus subtilis*, closely related species thereof, and constructions useful for production of biotin. (See page 2, lines 44-53). In Bower(EP), the enzymatic steps from pimelyl-CoA (PmCoA) to biotin are disclosed. (See page 2, lines 5-19 and FIG. 1). The step from 7-keto-8-amino pelargonic acid ("KAPA") to 7,8-diamino-pelargonic acid ("DAPA") is disclosed to be mediated by DAPA amino transferase (bioA). (See page 2, lines 8-10). In FIG. 1, this step is shown to require S-adenosyl methionine (SAM).

In making the rejection, the Examiner contended that "Bower *et al.* teaches the *B. subtilis* strain B1282" and "strain *E. coli* MM294," both of which express a "lysine-utilizing DAPA aminotransferase" and are cultivated in media that contains "at least 10 mM lysine" (Paper No. 33 at 10).

As is well settled, anticipation requires "identity of invention." *Glaverbel Societe Anonyme v. Northlake Mktg. & Supply*, 33 USPQ2d 1496, 1498 (Fed. Cir. 1995). **Each and every element recited in a claim** must be found in a single prior art reference and arranged as in the claim. *In re Marshall*, 198 USPQ 344, 346 (CCPA 1978); *Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Co.*, 221 USPQ 481, 485 (Fed. Cir. 1984). Furthermore, in a §102(b) rejection there must be **no difference** between what is claimed and what is disclosed in the applied reference. *In re Kalm*, 154 USPQ 10, 12 (CCPA 1967); *Scripps v. Genentech Inc.*, 18 USPQ2d 1001, 1010 (Fed. Cir. 1991). "Moreover, it is incumbent upon the Examiner to **identify**

wherein each and every facet of the claimed invention is disclosed in the applied reference." *Ex parte Levy*, 17 USPQ2d 1461, 1462 (BPAI 1990). And also, "***all words in a claim must be considered*** in judging the patentability of that claim against the prior art." *In re Wilson*, 165 USPQ 494, 496 (1970).

Initially, we note that because the claims are ***method claims***, the Examiner is required to show each and every element of the recited method. All of the rejected claims recite a step wherein lysine, a lysine analog, or a lysine precursor is "***added to the culture***." (See e.g., claim 1). The rejection fails to identify where in Bower(EP) is disclosed that lysine, a lysine analog, or a lysine precursor is added to the bacterial culture. Accordingly, it is respectfully submitted that rejection fails to identify where in Bower(EP) is disclosed ***each and every element*** of the recited method. For this reason alone, the rejection should be withdrawn.

Further, claim 1, as amended, recites that "***culturing takes place*** in an environment wherein lysine, a lysine analog, or a lysine precursor is ***exogenously added to the culture***" and further, that the lysine is present "***during the entire culturing step***." As is apparent from the specification, lysine is actively fed to fermenting cultures of bacteria. (See page 17, line 22 to page 19, line 26; page 21, line 1 to page 22, line 13; and Tables 3, 5A, 5B and 6). Further, as can be seen from the data supplied in Table 3 (compare lysine in feed and batch to only in batch), optimal results are only obtained when culturing takes place in an environment wherein lysine is exogenously added to the culture and wherein lysine is present during the entire culturing step. It is respectfully submitted that the rejection fails to identify where in Bower(EP) is disclosed that "***culturing takes place*** in an environment wherein lysine, a

lysine analog, or a lysine precursor is **exogenously added to the culture**," and that the lysine is present "**during the entire culturing step**," however, this was the Examiner's burden. It is respectfully submitted that for this reason also, the rejection should be withdrawn.

§102(e) Anticipation Rejection

Claims 1, 3, 7 and 11-22 were rejected under 35 U.S.C. §102(e) as being anticipated by Bower *et al.* (U.S. Patent No. 6,057,136) ("Bower(US)"). (Paper No. 33 at 11-12).

Bower(US) discloses genes of the biotin biosynthetic operon of *Bacillus subtilis*, closely related species thereof, and constructions useful for production of biotin. (See Abstract). In Bower, the enzymatic steps from pimelyl-CoA (PmCoA) to biotin are disclosed. (See Col. 1, lines 17-43 and FIG. 1). The step from 7-keto-8-amino pelargonic acid ("KAPA") to 7,8-diamino-pelargonic acid ("DAPA") is disclosed to be mediated by DAPA amino transferase (*bioA*). (See Col. 1, lines 25-26). In FIG. 1, this step is shown to require S-adenosyl methionine (SAM).

Because the disclosures of Bower(EP) and Bower(US) are substantially similar, it is submitted that the same arguments set forth above for the §102(b) rejection over Bower(EP) also apply to the §102(e) rejection over Bower(US).

In short, the rejection fails to identify where in Bower(US) is disclosed that lysine, a lysine analog, or a lysine precursor is "**added to the bacterial culture**." Further, the rejection fails to identify where in Bower(US) is disclosed that "**culturing takes place**" in an environment wherein lysine, a lysine analog, or a lysine precursor is

exogenously added to the culture" and that lysine is present "*during the entire culturing step.*" In view of the foregoing, it is respectfully submitted that the rejection fails to identify where in Bower(US) is disclosed ***each and every element*** of the recited method. Accordingly, withdrawal of the rejection is respectfully requested.

With respect to the Examiner's comments in ¶21 of the Office Action (paper No. 33 at 12), we note that, as set forth above, the critical element missing from the Examiner's analysis is that the claims recite that the lysine, lysine analog, or lysine precursor is added to a "***bacterial culture***", not merely to the pre-existing media, and further that "***culturing takes place*** in an environment wherein lysine, a lysine analog, or a lysine precursor is ***exogenously added to the culture,***" and not merely that there is lysine present in the initial media.

For the reasons set forth above, it is respectfully submitted that the rejection should be withdrawn.

§103(a) Obviousness Rejection

Claims 8 and 20 were rejected under 35 U.S.C. §103(a) as being unpatentable over Bower(EP) in view of Yamada *et al.* (U.S. Patent No. 4,563,426) ("Yamada"). (Paper No. 33 at 14-15).

The disclosure of Bower(EP) is set forth above.

Yamada discloses a process for cultivating "a microorganism having the ability to produce a biotin-vitamer in a culture medium in the presence of a biotin-vitamer precursor to produce the biotin-vitamer and accumulate it in the culture medium." (Col. 1, Ins. 23-30). The biotin-vitamer precursor is "added to the culture

medium after the microbial cells have grown." (Col. 1, Ins. 30-32). The biotin-vitamer produced by the disclosed process "consists mainly of dethiobiotin, biotin, and biotin sulfoxide." (Col. 1, Ins. 33-35). Yamada disclose that a biotin-vitamer precursor is "a precursor of the desired biotin-vitamer," such as, for example, pimelic acid, azelaic acid, and desthiobiotin. (Col. 1, Ins. 58-66). Nitrogen sources in the culture medium include, *inter alia*, case amino acids and amino acids. (Col. 2, Ins. 4-9).

In making the rejection, the Examiner asserted that Bower(EP) teaches "dethiobiotin made by bacteria." (Paper No. 33 at 14). The Examiner acknowledged, however, that Bower(EP) differs from the presently claimed invention in that "Bower *et al.* does not teach the conversion of dethiobiotin produced by bacteria into biotin by a separate fermentation." (*Id.*). The Examiner also acknowledged that "Yamada *et al.* does not teach dethiobiotin production by recombinant *B. subtilis* or *E. coli*." (*Id.*).

To fill the acknowledged gap, the Examiner relied upon Yamada as teaching the conversion of "dethiobiotin into biotin through a fermentation process." (*Id.*). The Examiner then concluded that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to convert dethiobiotin into biotin through a fermentation process, as taught by Yamada *et al.*, with dethiobiotin made by bacteria, as taught by Bower *et al.*" (*Id.*).

As is fundamental, all properties and attributes of a claimed method must be considered by the Examiner. Accordingly, the arguments presented above with respect to the anticipation rejection apply to the obviousness rejection as well. We note that claims 8 and 20 depend from claim 1 or claim 2 (as well as claims 3 and 4, which also depend from claims 1 and 2). As set forth in detail above, amended claim 1 recites

that "**culturing takes place** in an environment wherein lysine, a lysine analog, or a lysine precursor is **exogenously added to the culture**" and that lysine is present "**during the entire culturing step.**" Further, we note that claim 2, as amended, recites culturing a bacterium "wherein the lysine biosynthetic pathway is deregulated in said bacterium." The rejection fails to identify where in Yamada or Bower(EP) such limitations are disclosed or suggested. Accordingly, for this reason alone, the rejection should be withdrawn.

We further note that the Examiner was required to demonstrate a suggestion which would have "strongly motivated" one to carry out the method as claimed. *Ex parte Graselli*, 231 USPQ 393, 394 (Bd. App. 1986). The type of motivation which would have "**impelled**" one to do so (*Ex parte Levengood*, 28 USPQ2d 1300, 1301-02 (BPAI 1993)), and the type of suggestion that the changes "**should**" be made. *Ex parte Markowitz*, 143 USPQ 303, 305 (Bd. App. 1964).

As is well accepted, "the genius of invention is often a combination of known elements which in hindsight seems preordained... [t]o prevent hindsight invalidation of patent claims, the law requires some 'teaching, suggestion or reason' to combine cited references." *Gambro Lundia AB v. Baxter Healthcare Corp.*, 42 USPQ2d 1378, 1383 (Fed. Cir. 1997). Consequently, the test of whether to combine references needs to be applied rigorously. See *In re Dembiczak*, 50 USPQ2d 1614, 1617 (Fed. Cir. 1999) ("guarding against falling victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher").

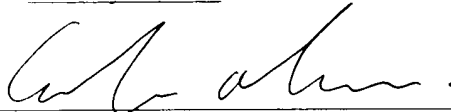
In making the rejection, the Examiner has only asserted that "[a] person of ordinary skill in the art is motivated to convert dethiobiotin into biotin using a

fermentation process because biotin is an essential vitamin for all organisms, therefore any process which can produce this vitamin in large amounts is highly desirable." (Paper No. 33 at 14). The Examiner has merely posited that the desired outcome of the claimed invention, a process which can produce biotin in large amounts, is proper motivation. Recognition of the solution to a problem, however, is not the kind of evidence required to combine documents. Rather, the motivation or suggestion to make the proposed combination must be found in the documents themselves or recognized in the art. Here, Bower(EP) already discloses that its process produces the "high level production of biotin." (See e.g., Abstract). The rejection provides no evidence that would suggest or motivate one to further modify Bower(EP) with Yamada with the expectation of producing even higher levels of biotin than disclosed for the Bower(EP) process. It is respectfully submitted that this is precisely the kind of hindsight analysis that the aforementioned cases warn against. For this reason also, the rejection should be withdrawn.

We also note that the claims as amended recite that at least 10mM lysine is present "**during the entire culturing step.**" The rejection does not identify where in either Bower(EP) or Yamada such a recitation is disclosed or suggested. As disclosed in the present specification it was unexpectedly discovered that maintaining the availability of lysine during the **entire** culturing step, especially during the feeding phase, resulted in a 10-fold increase in biotin production (See e.g., page 11, line 31 to page 12, line 1) that was neither disclosed nor suggested by Bower(EP) alone or in combination with Yamada. For this reason, as well, the rejection should be withdrawn.

Favorable action on the merits including entry of the amendments and the drawing corrections, withdrawal of the rejections, and allowance of all the claims, respectfully, is requested. If the Examiner has any questions regarding this paper, please contact the undersigned attorney.

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner for Patents, Washington, D.C. 20231, on February 13, 2003.



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EXHIBIT 2

"Marked Up" Amendments to Claims Pursuant to Rule 1.121(c)(1)(ii)

1. (Twice amended) A method of producing a biotin vitamer by:
 - (a) culturing a bacterium comprising a lysine-utilizing diaminopelargonic acid (DAPA) aminotransferase, said culturing taking place in an environment wherein lysine, a lysine analog, or a lysine precursor is exogenously added to the culture to provide a concentration of [and totals] at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step; and
 - (b) recovering said biotin vitamer.

2. (Amended) A method of producing a biotin vitamer by:
 - (a) culturing a bacterium comprising a lysine-utilizing DAPA aminotransferase, wherein the lysine biosynthetic pathway is deregulated in said bacterium [is deregulated with respect to lysine production]; and
 - (b) recovering said biotin vitamer.

6. (Twice amended) The method of claim 2 or claim 4, in which lysine, a lysine analog, or a lysine precursor is exogenously added to the culture to provide a concentration of [and totals] at least 10 mmoles lysine, lysine analog, or lysine precursor per liter of culture during the entire culturing step.
11. (Amended) The method of claim 1 or claim 2, wherein [in which the bacterium is deregulated with respect to] at least one biotin synthetic pathway step in addition to [bioA] expression of the bioA gene is deregulated in said bacterium.
13. (Amended) The method of claim 1, claim 2, claim 3, or claim 4, wherein said bacterium is further engineered to produce a S-adenosylmethionine (SAM)-utilizing DAPA aminotransferase.
17. (Amended) The method of claim 15 in which lysine or a lysine analog exogenously added to the culture provides a concentration of [totals] at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step.

**OVERCOMING DAPA AMINOTRANSFERASE BOTTLENECKS IN
BIOTIN VITAMERS BIOSYNTHESIS**

18. (Amended) The method of claim 16 in which lysine or a lysine analog exogenously added to the culture provides a concentration of [totals] at least 10 mmoles lysine or lysine analog per liter of culture during the entire culturing step.
21. (Amended) The method of claim 13 wherein [in which the bacterium is deregulated with respect to] at least one biotin synthetic pathway step other than [*bioA*] expression of the *bioA* gene is deregulated in said bacterium.